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2

Macrophage biochemistry, activation and function

M. D. KASTELLO and P. G. CANONICO

Department of Antiviral Studies, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701, U.S.A.

CONTENTS

I. INTRODUCTION

Functionally and morphologically heterogeneous macrophages are widely scattered in body tissues. They are represented by the alveolar macrophages of the lung, Kupffer cells of the liver, cells found free in the pleural and peritoneal cavities and cells lining the sinusoids of the spleen and bone marrow. This varied group of phagocytic cells, along with monocytes, compose the mononuclear phagocyte system (Nelson, 1976).

These phagocytes have their origin in the granulocytic series of the bone marrow where the promonocyte is the earliest recognizable form (Van Furth and Cohn, 1968; Van Furth, 1970). This stem cell actively divides and with maturation gives rise to the monocyte. After a short-lived residence in the bone marrow, monocytes are released into the blood where they normally account for 3—5% of the circulating leukocytes. They then leave the circulation, enter the tissues (Meuret et al., 1975) and mature into tissue macrophages. In the process, they increase in size and their mitochondria and components of the vacuolar apparatus become more abundant. Functional capabilities, including phagocytic activity, protein synthesis and surface receptors, also increase (Cline et al., 1978).

The fully mature macrophage is capable of participating in varied biological and immunological activities, including protecting the tissues against infection by invading microorganisms. In infections, the severity of illness and the ultimate survival of the host depend greatly on how effectively macrophages can perform their microbicidal function. To wage an effective battle against potential pathogens, macrophages undergo a further transformation of their biochemical and physiological properties designed to enhance their microbicidal capacity. The mechanism underlying the enhancement of macrophage functions during infection serves as the theme of this chapter.

In the following section we present a discussion of the biochemical makeup of macrophages as revealed by analytical cell fractionation methods. The goal of this investigative approach is to obtain a map of the manner in which enzymes and other biochemical constituents are distributed between cellular organelles. This information is useful in evaluating subcellular events which contribute to the biological functions of macrophages in disease and therapeutic processes. For example, cellular fractionation led Lowrie et al. (1979) to conclude that normal and Bacillus Calmette-Guérin (BCG)-induced rabbit lung macrophages have three different types of hydrolase-containing granules. They also demonstrated that the granules of BCG-induced macrophages undergo striking changes in their physical properties. Cellular fractionation can provide quantitative information as to the intracellular compartments where microorganisms become sequestered following phagocytosis (Canonico, 1980; Little et al., 1980). The intracellular localization and concentration of antimicrobial agents can also be obtained by this approach (Tulkens and Trouet, 1978).

II. BIOCHEMICAL CYTOLOGY

A. *Perspective*

The range of distribution, morphology and function of macrophages in different

TABLE 1

MACROPHAGE ENZYMES[a]

Enzyme	Macrophage activity[b] — references[c]		
	Peritoneal	Alveolar	Other
Ectoenzymes			
5'-Nucleotidase	+2, 3, 8, 16		±6
Leucine aminopeptidase	+26		+6
Alkaline phosphodiesterase	+11		
Alkaline phosphatase	+26		
ATPase	+16		
p-Nitrophenylphosphatase	+16		
NADase	+2		
Lysomal enzymes			
Peroxidase	−12, 16	−25	±10, 17
β-Glucuronidase	+7—9, 13, 22	+9, 27	+4, 18
N-Acetyl-β-glucosaminidase	+7, 8, 22		+23
Cathepsin D	+9, 20	+9	+5, 23
Arylsulfatase B	+13		+17
α-Mannosidase	+8, 22		
Acid phosphatase	+2, 3, 9, 13, 22	+9, 27	+17
Acid DNAase			+4
β-Galactosidase	+21		+18
Acid RNAase	+9	+9	
Lipase	+9	+9	
α-Galactosidase	+8		
Urea cycle enzymes			
Carbamyl-phosphate synthase	+15	+15	+15
Ornithine carbamoyltransferase	+15	+15	+15
Argininosuccinate synthetase	+15	+15	+15
Argininosuccinate lyase	+15	+15	+15
Arginase	+15	+15	+15
Ornithine—oxo-acid transaminase	+15		
Neutral proteinases			
Collagenase	+28		
Elastase	+29, 30	+3	+6
Plasminogen activator	−19, 24		+19
Caseinase	+1		
Other enzymes			
Lysozyme	+3	+	+6
Sialyltransferase		+1[d]	
α_3-Fucosyltransferase		+1[e]	
Galactosyltransferase	+8	+14	
N-Acetylglucosaminyltransferase	+8		
Lactate dehydrogenase	+8		
Malate dehydrogenase	+8		
Cytochrome oxidase	+8		
Alkaline α-glucosidase	+8		
Sulfatase c	+8		

[a] Modified from Morahan, 1980.

[b] (+)Exhibits activity; (−) lacks activity; (±) weak or marginal activity.

[c] References: (1) Adams, 1978; (2) Artman and Seeley, 1979; (3) Bar-Shavit et al., 1979; (4) Berg and Boman, 1973; (5) Berg and Munthe-Kaas, 1977; (6) Bonney et al., 1978a; (7) Bonney et al., 1978b; (8) Canonico et al., 1978; (9) Cohn and Wiener, 1963a; (10) Crofton et al., 1978; (11) Edelson and Erbs, 1978; (12) Edelson et al., 1975; (13) Hard, 1970; (14) Hofmann et al., 1978a; (15) Hofmann et al., 1978b; (16) Karnovsky et al., 1975; (17) Knook et al., 1977; (18) Knook and Sleyster, 1976; (19) Lin and Gordon, 1979; (20) Mørland and Kaplan, 1977; (21) Pedersen et al., 1979; (22) Schnyder and Baggiolini, 1978 (23) Sleyster et al., 1977; (24) Unkeless et al., 1974; (25) Van Oud Alblas and Van Furth, 1979; (26) Wachsmuth, 1975; (27) Warr et al., 1979; (28) Werb and Gordon, 1975a; (29) Werb and Gordon, 1975b; (30) White et al., 1977.

tissues are reflected by the variations in biochemical properties among different populations of phagocytes. A limited selection of an increasing list of enzymes associated with resident macrophages from various tissues is presented in Tables 1 and 2 (See Morahan, 1980, for a more extensive review). An appreciation of the biochemical composition of macrophages undoubtedly can assist our understanding of mechanisms which regulate expression of biological functions. Often, there is also a requirement to understand the biochemical and physical characteristics of the organelles that ultimately determine the capacity of macrophages to perform their biologic roles. Fractionation and structure-linked functional studies on macrophages from various tissues were initiated by Cohn and Wiener (1963a) who illustrated the relevance of such investigations in studying the phagocytic activity of BCG-induced alveolar macrophages (Cohn and Wiener, 1963b).

Unlike some other types of cells, macrophages are not readily obtained in large quantities from experimental laboratory animals. This single factor has in large measure limited the application of cellular fractionation techniques for deciphering the complex mechanisms which govern macrophage physiology. Some workers increase the yield of peritoneal macrophages by prior injection of inflammatory agents into the peritoneal cavity (Cohn, 1974). Such procedures, however, elicit a population of phagocytes having altered physical and biochemical properties (Wiener and Curelaru, 1975). Fractionation of cellular homogenates by differential and isopycnic centrifugation has shown that the lysosomal enzymes of elicited macrophages are associated with particles of varying physical properties. In the different types of particles, the ratios of the various acid hydrolase activities appear to be dissimilar (Wiener and Curelaru, 1975).

Only recently has the characterization of subcellular components been accomplished from nonelicited resident and in vitro cultivated populations of mouse peritoneal macrophages. These studies have been made possible due to the development of sensitive fluoro- and radio-metric enzyme assays which permit the biochemical characterization of subcellular components of relatively small quantities of cells. Cytoplasmic extracts containing 2—4 mg of protein from the homogenization of macrophages collected from the peritoneum of 1—2 mice can be subjected to analytical fractionation by isopycnic centrifugation methods (Canonico et al., 1978). Results from these studies have led to recognition of three characteristic equilibrium density patterns for constituents of cellular components (Fig. 1). Comparative analysis of these data with known physical and biochemical properties of cell components of other tissues has led to the following assignment of marker enzymes to specific macrophage components. This assessment is summarized in Table 3.

B. *Mitochondria*

The nearly identical, narrow, symmetrical distribution patterns of cytochrome

TABLE 2

SPECIFIC AND RELATIVE CONTENT OF MEASURED CONSTITUENTS IN HOMOGENATES OF CULTURED MOUSE PLASMA MEMBRANES

Constituent	Mean specific content[a] ±S.E.				P^b	Relative total content of 72-h cultures in multiples of initial content
	1-h culture		72-h culture			
Protein (μg/μg DNA)	61 ± 5.8	(4)	238 ± 5	(5)	0.001	3.9
Lactate dehydrogenase			520 ±59	(9)	—	
5′-Nucleotidase	42.5 ± 3.6	(3)	72.1 ± 4.8	(8)	0.01	6.6
Galactosyltransferase	0.076± 0.002	(3)	0.100 ± 0.007	(8)	n.s.	5.1
N-Acetylglucosaminyltransferase			0.0039± 0.002	(5)	—	
Cytochrome oxidase	8.50 ± 0.48	(4)	9.77 ± 1.12	(10)	n.s.	4.5
Malate dehydrogenase	767 ±34	(3)	512 ±43	(9)	0.01	2.6
Alkaline α-glucosidase	0.992± 0.037	(4)	0.946 ± 0.056	(9)	n.s.	3.7
Sulfatase c	0.226± 0.018	(4)	0.468 ± 0.029	(11)	0.001	8.1
α-Galactosidase	0.953± 0.555	(4)	9.69 ± 0.75	(11)	0.001	39.7
N-Acetyl-β-glucosaminidase	73.4 ± 3.5	(4)	93.7 ± 4.5	(9)	0.02	5.0
α-Mannosidase	4.72 ± 0.57	(4)	23.0 ± 1.8	(10)	0.001	19.0
β-Glucuronidase	1.12		5.33 ± 0.12	(5)		18.6
Acid phosphatase			40.9 ± 3.0	(8)		
Cholesterol (μg/mg protein)			6.06 ± 0.37	(8)		

[a] Figures in parentheses refer to number of experiments.
[b] Statistical significance of the differences; n.s. not significant.
Data from Canonico et al., 1978. All enzyme contents expressed as mU/mg protein.

TABLE 3

SUMMARY OF PHYSICAL PROPERTIES OF MACROPHAGE ENZYMES AND THEIR PROPOSED INTRACELLULAR LOCATION

Density distribution of sedimentable activity		Structural latency	Sensitivity to solubilization[a]		Enzyme	Proposed intracellular location
1-h culture	72-h culture		Digitonin (0.012%)	Triton X-100 (0.22%)		
Narrow symmetrical pattern with mode at 1.18	no change	n.s.[b]	n.t.	n.t.	cytochrome oxidase	mitochondria
		prevalent	0	0	malate dehydrogenase	
Broad pattern centered around modal density at 1.20	shifted to higher density	prevalent	++	++	α-mannosidase	lysosomes
		prevalent	+	++	α-galactosidase	
		prevalent	+	++	*N*-acetyl-β-glucosaminidase	
		n.s.[b]	n.t.	n.t.	β-glucuronidase	
	no change	none	0	++	sulfatase	endoplasmic reticulum
		none	0	++	alkaline α-glucosidase	
Skewed patterns with modes at 1.14—1.16	no change	minimal	0	+	5′-nucleotidase	plasma membrane and/or Golgi
		n.s.[b]	n.t.	n.t.	galactosyltransferase	
		n.s.[b]	n.t.	n.t.	*N*-acetylglucosaminyl-transferase	

[a] (0)Indicates solubilization of < 10%, (+) 10—30%; (++) > 30%. n.t., not tested

[b] Detergents required for maximal expression of activity (data not shown).

Data from Canonico et al., 1978.

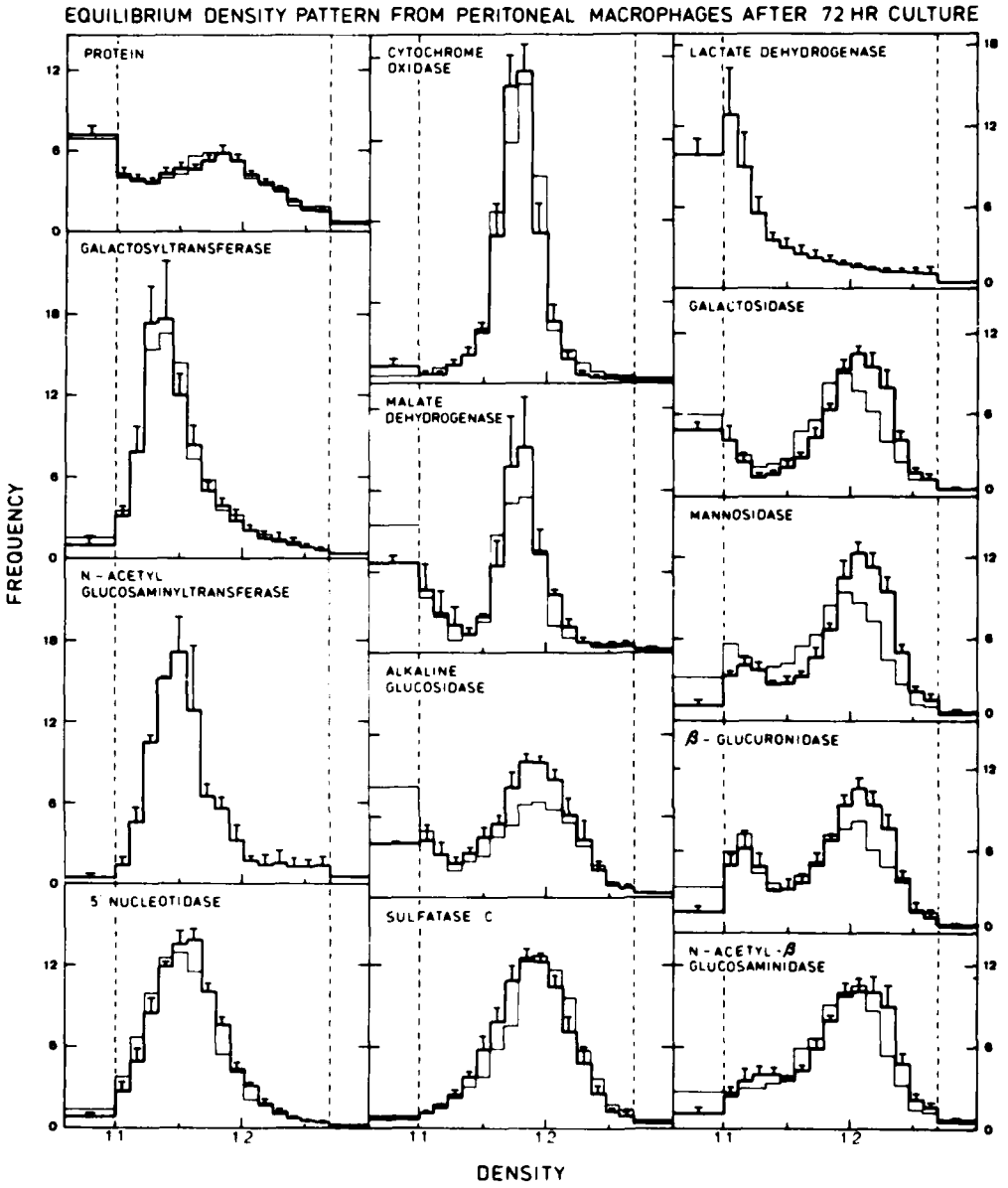

Fig. 1. Distribution profiles of constituents from cytoplasmic extracts of cultivated mouse macrophages after isopycnic equilibration in linear sucrose gradient (thick line). Thin lines show distribution profiles obtained from resident macrophages. Results are presented in the form of normalized and averaged frequency histograms. The density scale, divided into 15 normalized fractions of identical density increment, extends from 1.10 to 1.27. The frequency represents $\Delta Q/(\Sigma Q \Delta \rho)$, where ΔQ is the amount of constituent present within the section, and ΣQ the sum of the amounts found in all the subfractions. The surface area of each histogram bar then gives the fractional amount of constituent present within each normalized fraction. Distribution profiles are flanked on either side by blocks arbitrarily constructed over the density spans 1.07—1.10 and 1.27—1.30 and refer to material recovered above and below the linear limits of the gradient. The total area of each histogram is then equal to 1. Diagrams show averages of results with S.E. Data from Canonico et al., 1978.

oxidase and the sedimentable portion of malate dehydrogenase reflect the distribution of mitochondria. In this respect, the cytochrome oxidase distribution from macrophages reflects the equilibrium density pattern for liver mitochondria (Beaufay et al., 1964). The distribution of cytochrome oxidase is mimicked by the sedimentable portion of malate dehydrogenase, which is also known to have a mitochondrial matrix localization in liver (Marco et al., 1969). Whether the activity of malate dehydrogenase in the soluble fraction can be ascribed fully to leakage from mitochondria, or partly to the occurrence of this enzyme in the cytosol, as shown in other tissues (Roodyn, 1975), is a pending question.

C. *Lysosomes*

The biochemical concept of lysosomes envisions lytic organelles which contain enzymes that are active at acid pH, exhibit structure-linked latency and are unmasked or solubilized in parallel fashion by treatment with detergents (De Duve et al., 1955; De Duve, 1963). In liver, lysosomes are polydisperse in density and, on an average, equilibrate at a relatively high density in sucrose gradients (Beaufay et al., 1964). α-Mannosidase, α-galactosidase, β-glucuronidase and *N*-acetyl-β-glucosaminidase of macrophages are found to share the above properties; it is therefore believed that they also characterize the lysosomes of macrophages.

The equilibrium density distribution profiles of lysosomal hydrolases obtained from cultivated macrophages clearly dissociate these enzymes from all other constituents. The profiles from resident cells, however, cannot be differentiated from the endoplasmic reticulum enzymes, sulfatase c and alkaline α-glucosidase. Dissociation of the lysosomal enzyme patterns from sulfatase c and alkaline α-glucosidase can be achieved by fractionation of macrophages loaded with Triton WR-1339. When fed to macrophages, this nondegradable detergent accumulates within lysosomes and decreases their density (Fig. 2).

Subtle differences among the density profiles of acid hydrolases obtained from resident macrophages and the varied extent by which their modal densities increase during in vitro culture imply the possible presence of two or more populations of lysosomes in resident cells. There is general agreement that blood monocytes emigrating from the circulation into tissues and body cavities mature into typical macrophages (Van Furth et al., 1972). This maturation from monocytes to macrophages is a continuous process (Nichols and Bainton 1975). Hence, at any particular point in time, the resident macrophages of the peritoneal cavity actually represent a heterogeneous population of mononuclear phagocytes at various stages of maturation and differentiation. Moreover, macrophages have been shown to produce two kinds of lysosomes (Nichols and Bainton, 1975). In monocytes, primary lysosomes take the form of large storage granules whereas in mature macrophages they are Golgi complex-derived, coated vesicles found after the granules have become depleted. The heterogeneity among density profiles of acid hydrolases from resident cells

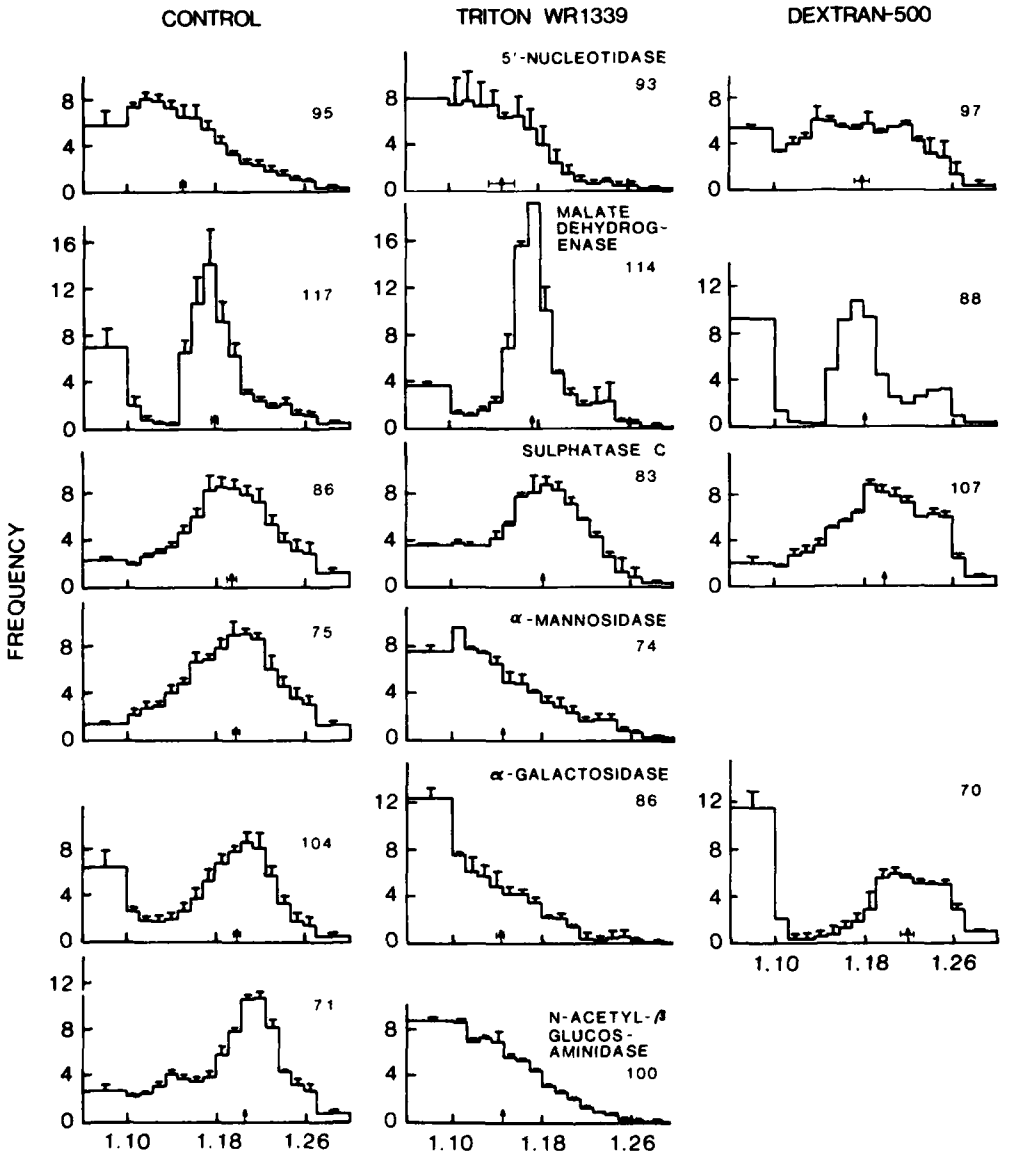

Fig. 2. Distribution profiles of constituents from cytoplasmic extracts of control (left), Triton WR-1339- (center) and Dextran 500-loaded (right) rat macrophages. Results are presented as described in Fig. 1.

possibly reflects differences in enzymatic and physical properties of lysosomes originating from a continuous spectrum of mononuclear phagocytes at different stages of maturity. In vitro culture conditions, on the other hand, restrict the constant infusion of less differentiated cells into the population. As the differentiation process becomes complete under the inductive effect of the culture conditions a more uniform population of mature macrophages is obtained. It is anticipated that such cells yield a more homogeneous population of lysosomes and display a greater uniformity in the density distribution profiles of their acid hydrolases.

D. *Plasma membranes and Golgi*

The bulk of the 5′-nucleotidase of liver and cultivated fibroblasts is found in the plasma membrane (DePierre and Karnovsky, 1973; Tulkens et al., 1974). The homogeneous equilibrium density profiles obtained for 5′-nucleotidase from resident and cultivated macrophages are similar to patterns obtained from other tissues (Beaufay et al., 1974; Shur and Roth, 1975) and suggest an almost exclusive plasma membrane localization of this enzyme on the macrophage. Huggins (personal communication) investigated this localization by labelling the surface of macrophages with radiolabeled wheat germ agglutinin prior to homogenization, and subsequently treating the homogenate with digitonin to increase the density of the cholesterol-containing plasma membrane. Following isopycnic centrifugation, the 5′-nucleotidase and wheat germ agglutinin coequilibrated at a denser region of the gradient, confirming the plasma membrane localization of 5′-nucleotidase.

The association of glycosyltransferases within Golgi apparatus also has been clearly established (Fleischer et al., 1969; Morre et al., 1969; Schachter et al., 1970). However, in macrophages, as in liver (Beaufay et al., 1974), the density distribution profiles for galactosyltransferase and *N*-acetylglucosaminyltransferase are not sufficiently unique to dissociate them from the plasma membrane marker, 5′-nucleotidase. Treatment of macrophage homogenates with digitonin causes a dissociation of 5′-nucleotidase from *N*-acetylglucosaminyltranferase and from a portion of galactosyltransferase (Huggins, personal communication). It appears that a part of the galactosyltransferase activity of macrophages is associated with the plasma membrane. The remaining portion of this enzyme and all of the *N*-aceltylglucosaminyltransferase are located in Golgi elements.

E. *Endoplasmic reticulum*

Glucose-6-phosphatase, esterase and other constitutents have served as markers for endoplasmic reticulum of rat liver (Amar-Costesec et al., 1974; Beaufay et al., 1974). Peritoneal macrophages are deficient or contain insuf-

ficient quantities of the classical constituents to be used as reliable markers. However, they show fair activity for both alkaline α-glucosidase and sulfatase c, which are also constituents of the microsomal fraction in liver (Dodgson et al., 1955; Lejeune et al., 1963) and presumed to be associated with the endoplasmic reticulum of hepatocytes. Alkaline α-glucosidase is also reported to be a microsomal enzyme in other tissues (Angelini and Engel, 1973; Peters and De Duve, 1974).

The density distribution profiles of sulfatase c and alkaline α-glucosidase of mouse macrophages are unique relative to the more complex profiles of liver endoplasmic reticulum constituents. The difference is not surprising considering the complex biochemical and morphologic organization of liver endoplasmic reticulum (Wibo et al., 1971; Beaufay et al., 1974). The conclusion that sulfatase c and alkaline α-glucosidase are constituents of the endoplasmic reticulum of peritoneal macrophages is derived from their density profiles, which clearly dissociate from those of the plasma membrane, Golgi and mitochondrial markers. Sulfatase c and alkaline α-glucosidase are differentiated from lysosomal markers on the basis that they do not show structure-linked latency and by the fact that their density profiles do not shift following in vitro culture or loading of macrophages with Triton WR-1339 or Dextran 500 (Fig. 2).

III. ACTIVATION

The macrophage is unique in that it appears to require functional modification before its antimicrobial capacity can be fully expressed. Under the influence of a variety of stimuli, macrophages undergo extensive adaptive changes which may include but are not limited to an increase in size, stimulation of protein synthesis and ruffling of the plasma membrane; an increased capacity for adhering to a substratum and occupation of an area that can be 8-times greater than that of their unspread state; an enhanced phagocytic and pinocytic capacity; an increased number of endocytic vesicles and phagolysosomes; a stimulation of metabolism, secretion of enzymes and increased microbicidal activity for a range of bacteria (Cohn, 1978; Karnovsky and Lazdins, 1978; North, 1978). It is reasonable to presume that this impressive list of adaptive changes enables macrophages to carry out their protective and homeostatic functions more efficiently. In vivo, this enhancement of macrophage function occurs initially at sites of inflammation. The entry and multiplication of microorganisms in the tissues, the subsequent tissue damage, the irritating effects of the organisms themselves and toxins produced by them evoke a sequence of vascular, humoral and cellular alterations which define the inflammatory response. The development of an inflammatory process is a dynamic process characterized by the continuous migration of macrophages into the lesion until all bacteria and necrotic tissue have been removed. This process represents a primitive universal response to tissue injury and occurs in lower animals without an

immune system. The inflammatory response serves not only to impede the further multiplication and dissemination of microbes, but in instances when local defenses are insufficient to contain the pathogen or its products, to trigger adaptive changes in phagocytes at distant tissue sites as well.

TABLE 4

RESISTANCE OF AKR/J MICE TO LISTERIA MONOCYTOGENES[a] INDUCED BY VARIOUS TREATMENTS

Treatment	% survivors by posttreatment day								
	1	2	3	6	9	12	20	30	60
Control	5.6	0	0	0	11	4.2	5.6	0	5.6
LVS (10^2 s.c.)	0	11	33.3	83.3	91.7	100	50	38.9	8.3
DPT (0.1 ml i.m.)	8.3	66.7	88.9	100	100	100	8.3	0	25
LVS (10^2 s.c.) + DPT (0.1 ml i.m.)	25	27.8	50	100	100	100	33.5	50	0
7.6% sodium caseinate (2 ml i.p.)	0	0	58.3	66.7	50	0	0	25	0
7.6% sodium caseinate (2 ml i.p.) + LVS (10^2 s.c.)	0	0	58.3	100	100	100	66.7	58.3	16.7

[a] Groups of AKR/J mice treated with the live vaccine strain (LVS) of *F. tularensis*, diphtheria-pertussis-tetanus toxoid vaccine (DPT) or sodium caseinate and appropriate control groups were challenged i.p. with lethal doses of *L. monocytogenes* (10^5 organisms) at various intervals (1—60 days) after treatment. Data from Eigelsbach, Hunter and Janssen (personal communication).

The net result is a population of macrophages which acquire a type of host defensive system which is antigenically nonspecific in its expression. Macrophages of hosts infected with one species of bacteria render the host more resistant to infection by a range of unrelated organisms. This concept is illustrated by data presented in Table 4: groups of AKR/J mice treated with *Francisella tularensis* (strain LVS), diphtheria-pertussis-tetanus toxoid (DPT) or caseinate develop nonspecific resistance against *Listeria monocytogenes* infection. This type of immunity is generally short-lived and expressed only in coincidence with the presence of an active inflammatory process.

The work of Mackaness (1964, 1970) and others introduced the concept of the specific activation of macrophages. It has led to an overwhelming amount of experimental evidence which shows that adaptive changes in macrophages that permit them to express enhanced microbicidal capacity can be mediated also by soluble factors secreted by specifically sensitized T lymphocytes. The evolution of the lymphoid system has therefore provided for an efficient, specific and long-lived system for the rapid and full activation of macrophages (North, 1978).

Attempts to understand the molecular basis of macrophage activation have

produced a substantial volume of literature on the differences between such cells and control macrophages. It appears, however, that the extent to which certain properties are expressed may differ, depending on the activating agent (Karnovsky and Lazdins, 1978; Morahan, 1980). These differences imply that macrophages have the capacity to respond differentially to various classes of activating molecules and have led to numerous discussions on the classification of activated macrophages (North, 1978; Karnovsky and Lazdins, 1978; Cohn, 1978; Morahan, 1980). It is possible, however, that the varied functional properties displayed by macrophages activated by different means may represent functional responses along a continuum, rather than a capacity for discrete responses to individual stimuli. Perhaps the type of response may be controlled, in part, by the environment of the inflammatory site and the age and maturity of the cell population. For example, hormones, trace metals and acute-phase serum protein levels show temporally related changes during infection and inflammation and could provide varied environments for macrophage activation depending upon the dynamics of the inflammatory response induced by different agents. Hence, the varied metabolic and functional states which different populations of activated macrophages are capable of expressing may be less significant than their potential to carry out the protective and homeostatic functions they are called upon to perform. These not only include phagocytic and microbicidal functions, but secretion of various proteins and mediators as well.

IV. FUNCTIONAL CONSEQUENCES OF MACROPHAGE ACTIVATION

A. *Regulation of host defense mechanisms*

Activated macrophages synthesize and release a number of products whose function is to regulate both specific and nonspecific host defense mechanisms. Endogenous pyrogen is one such product that is synthesized and released by activated macrophages. It is a polypeptide with a minimum molecular mass of about 15 000 daltons which initiates the hypothalamic events that lead to fever (Dinarello, 1980). In addition to granulocytes, endogenous pyrogen is produced and released by blood monocytes (Bodel and Atkins, 1967), alveolar (Atkins et al., 1967), peritoneal (Hahn et al., 1967) and fixed tissue macrophages (Dinarello et al., 1968). Interferon secretion is another important function of activated macrophages in host defense. Virus-infected cells can produce and secrete interferon within 2—6 h after virus adsorption through transcription and translational-requiring pathways (Page et al., 1978). Activated macrophages also produce a number of complement components including factor B, a component of the alternative pathway of complement activation. It is believed that the overall contribution of macrophages to circulating levels of complement

components in a host may be insignificant. On the other hand, their release at sites of inflammation may be important in the initiation of chemotaxis, cell proliferation or lysis, and secretion of cellular products (Davies and Allison, 1976; Page et al., 1978). Appropriately activated macrophages also release a battery of soluble factors which stimulate proliferation and differentiation of lymphoid cells (Diamantstein et al., 1979).

Activated macrophages may synthesize and secrete several enzymes which are active at neutral pH and play important roles in both the destructive and reparative phases of the inflammatory process (Davies and Allison, 1976). Collagenase, elastase and plasminogen activator are secreted from macrophages following stimulation by both bacterial constituents and products of immunological reactions. Acting on their natural substrates, they can bring about a remodeling of the intracellular matrix. Macrophages may be also selectively stimulated to release acid hydrolases. An appropriate extracellular environment can induce a striking increase in the intracellular level of acid hydrolases. However, the release of these enzymes extracellularly can be extremely selective. Ingestion of zymogen by macrophages, for example, results in the release of acid hydrolases; but endocytosis of sheep red blood cells does not induce this selective release (Weissman et al., 1971). There appears to a correlation between the capacity of substances to cause chronic inflammation and to induce release of lysosomal enzymes from macrophages in culture (Davies and Allison, 1976). Such substances may cause the extracellular release of lysosomal enzymes in sufficient quantities to produce damage to the host. Although tissue necrosis is not a desirable response by the host, at least in some situations, necrosis and the accompanying local biochemical changes may serve to impede further multiplication of microorganisms and act ultimately as a host defense system.

An important modifier of the extracellular activity of lysosomal enzymes is the various inhibitors present in tissue fluids. A number of proteinase inhibitors such as α_1-trypsin inhibitor and α_2-macroglobulin occur in blood and are known to penetrate into extravascular spaces in variable quantities. The balance between the concentration of inhibitors and proteinases may determine the relative activity of these enzymes at inflammatory sites (Davies and Allison, 1976).

It appears that the requirements for secretion of neutral proteinases by macrophages differ from those of acid hydrolases. Davies and Allison (1976) suggested that acid hydrolases and neutral proteinases are released at different stages in the life of a macrophage participating in the development of an inflammatory lesion. Acid hydrolases may be released early during the destructive phase of the process, while secretion of neutral proteinases may be more prominent during the reparative phase where they modulate the repair process.

Until recently, urea formation from NH_4^+ and HCO_3^- has been considered a specific function of mammalian liver. The work of Hofmann et al. (1978b) has

established that both peritoneal and bone marrow macrophages contain a complete urea cycle where arginosuccinate synthetase appears to be the rate-limiting enzyme in both types of macrophages. An important observation is that a large quantity of arginase is found not only in peritoneal and bone marrow macrophages, but in alveolar macrophages as well. Appropriately activated macrophages in culture and in vivo appear to secrete substantial amounts of arginase, which has the effect of depleting arginine from the culture media or in the microenvironment of a macrophage-rich inflammatory lesion. Arginine depletion due to arginase release from activated macrophages is believed to represent an important macrophage effector mechanism against a variety of targets such as malignant cells, virus-infected cells and microorganisms due to lethal deprivation of L-arginine (Currie et al., 1979).

Other enzymes shown to be secreted by macrophages are alkaline α-glucosidase and two glycosyltransferases, galactosyltransferase and *N*-acetylglucosaminyltransferase. The physiologic significance of the release of these enzymes is not readily apparent. Endogenous substrates for alkaline α-glucosidase which may be encountered by macrophages are limited. Glucosyl residues found on collagen and the complement component, Clq, are two possible endogenous substrates. Certain bacteria contain glucosyl residues on their cell wall and could represent a source of specific substrate for the secreted enzyme (Salton, 1960). It is unclear, however, if deglucosylation of collagen, Clq and certain microorganisms has any physiologic or microbicidal consequences.

With respect to the glycosyltransferases, it has been proposed that glycosylation of cell surface receptors may represent specific mechanisms of cell recognition and induction phenomena (Porter and Bernacki, 1975; Cacan et al., 1976). It is not inconsistent with this concept, therefore, to suggest that the extracellular release of galactosyltransferase and *N*-acetylglucosaminyltransferase may represent purposeful secretions, which at specific inflammatory or immunologic sites may modify the surface properties of adjacent cells and regulate or initiate various aspects of tissue repair or immunologic interactions.

B. *Enhancement of microbicidal functions*

Although the secretory functions of macrophages have received considerable attention for their role in the physiology and regulation of host responses in chronic inflammation, the destruction of invading microorganisms remains the primary function of macrophages in healthy hosts. Their activation must be viewed primarily as a mechanism to limit more efficiently the replication and dissemination of invading microorganisms. Important consequences of activation, therefore, include enhanced chemotactic and phagocytic capacity, engagement of metabolic pathways to meet increased energy demands and production of microbicidal products. These adaptations are generally sufficient to limit the

invasion of most microorganisms. However, a number of pathogens have developed the ability to survive and grow in macrophages. They have, in fact, learned to 'coax' the macrophage to protect, nourish and disseminate them to distant sites within the host (Mims, 1976).

Insight into mechanisms of macrophage microbicidal functions can be gained by knowledge of the ways virulent microorganisms have developed to permit their survival and replication within macrophages. It is with such information that molecular processes controlling macrophage activation can be uncovered, and agents for the nonspecific activation of macrophages can be developed.

At present, there are only a limited number of recognized requirements for the intracellular survival of microbial pathogens (Hart, 1979). Briefly, pathogens must evade the killing properties of activated oxygen intermediates and the degradative action of acid hydrolases. In addition, their physiological requirements must be suitably designed to permit growth and replication within various intracellular compartments. Resistance to activated oxygen intermediates may be a universal requirement for intracellular survival. Activation of macrophages coincident with phagocytosis or exposure to activating agents leads to the generation of the highly reactive and toxic free radical superoxide anion O_2^-, which behaves both as a powerful oxidant and as a reducing agent. In its behaviour as an oxidant, O_2^- is reduced to H_2O_2 . O_2^- also reacts with H_2O_2 to yield OH^-, $OH^\cdot$ and O_2 (Goren, 1977). Appropriate activation of macrophages results in the extracellular release of H_2O_2 and or $^1O_2$. It is presumed that activated oxygen intermediates are also delivered to intravacuolar sites harboring ingested pathogens. Successful pathogens, however, have found ways to resist these toxic moieties. Some virulent organisms have acquired endogenous catalase which is presumably used to decrease the concentration of H_2O_2 to nontoxic levels within their immediate intravacuolar environment. The elegant work of Nathan et al. (1979), Murray and Cohn (1979) and Murray et al. (1979) with *Trypanosoma cruzi* and *Toxoplasma gondii* indicates that, for certain pathogens, there may be a threshold in terms of the amount or concentration of activated oxygen intermediates necessary to produce inhibition or killing. In vivo, activation of macrophages by specific immunological products may be required to produce oxidative metabolites above the critical level required for full expression of the macrophages' microbicidal activity against these intracellular pathogens (Nathan et al., 1979).

In addition to a defense against toxic oxygen moieties, a successful intracellular pathogen must protect itself against the degradative action of acid hydrolases and the acid milieu of the lysosome. Some organisms have discovered means of preventing the fusion of phagocytic vesicles with lysosomes. The exact mechanism by which chlamydia, *Mycobacterium tuberculosis, Mycobacterium microti* and *T. gondii* prevent phagolysosome formation is unknown. However, an inhibition of phagosome-lysosome fusion by cyclic nucleotides produced from within the phagosome has been proposed (Lowrie et al., 1975; Goren, 1977).

TABLE 5

FATE OF *F. TULARENSIS* AFTER IN VITRO INFECTION OF RAT PERITONEAL MACROPHAGES

Strain	Serum	Bacteria/macrophage ratio		% of T_1
		1 h	18 h	
LVS[a]	normal	0.2	< 0.1	< 50
SCHU S4[b]	normal	0.4	3.5	875
LVS	immune	6.0	0.6	10
SCHU S4	immune	13.4	73.0	544

[a]Attenuated strain.
[b]Virulent strain.

Certain microorganisms such as *Mycobacterium leprae, L. monocytogenes* and *Salmonella typhimurium* do not prevent phagolysosome formation; rather they appear fully capable of resisting degradation by lysosomal enzymes and can grow and replicate within the acid environment of the phagolysosome (Hart, 1979). One organism belonging to this group which has been recently studied is *F. tularensis*. Both the virulent SCHU S4 strain and the attenuated live vaccine strain, LVS, when coated with specific antibodies are ingested and sequestered within phagolysosomes of rat peritoneal macrophages in culture (Canonico, 1980). In this environment, the virulent strain survives and grows but the attenuated strain is killed (Table 5). Both strains are equally resistant to activated oxygen metabolites generated by a hypoxanthine-xanthine oxidase system (Fig. 3). The virulent strain, however, appears better adapted for survival in the acid environment of the lysosome than LVS. SCHU S4 can synthesize protein and RNA optimally at pH 4.5, the expected pH of phagolysosomes (Ohkuma and Poole, 1978), but a more alkaline environment is required for optimal synthesis of macromolecules by LVS (Fig. 4). Furthermore, viability of the SCHU S4 strain is not altered by incubation of the bacteria with lysosomal enzymes at pH 4.5 (Fig. 5). Incubation of LVS under similar conditions leads to loss of viability.

It appears, therefore, that the SCHU S4 strain survives in phagolysosomes because it is resistant to toxic oxygen intermediates and acid hydrolases as well as being adapted for growth at acid pH. The attenuated strain, although resistant to oxygen metabolites, fails to grow intracellularly because its viability is compromised by its susceptibility to acid hydrolases and the acid environment of the phagolysosome. These conclusions are supported by the observation that increasing the pH of the phagolysosome by cultivating infected macrophages in the presence of a weak base results in intralysosomal growth and survival of the attenuated strain.

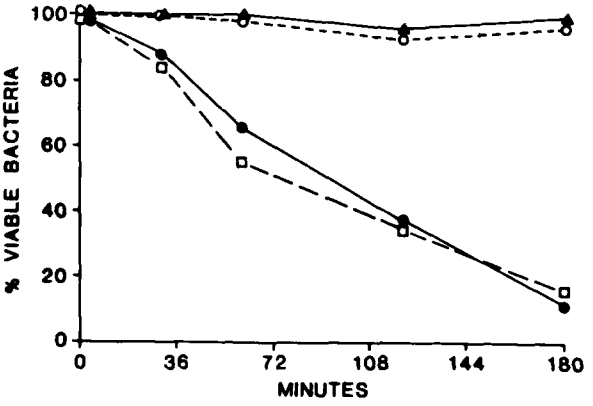

Fig. 3. In vitro effects of superoxide on bacterial killing. A hypoxanthine-xanthine oxidase system is used for the in vitro generation of activated oxygen radicals. The assay mixture consists of 15 mM phosphate buffer, pH 7.4, with 0.1% gelatin and 0.9% NaCl, 1 mM EDTA, 2.5 mM hypoxanthine, 25 μg (50 mU) xanthine oxidase and $2 \cdot 10^6$ test bacteria. Following incubation at 25°C for appropriate intervals, aliquots are obtained and the number of viable bacteria determined by plating. ▲, SCHU 24; ○, LVS; □, *S. aureus*; ●, *E. coli*.

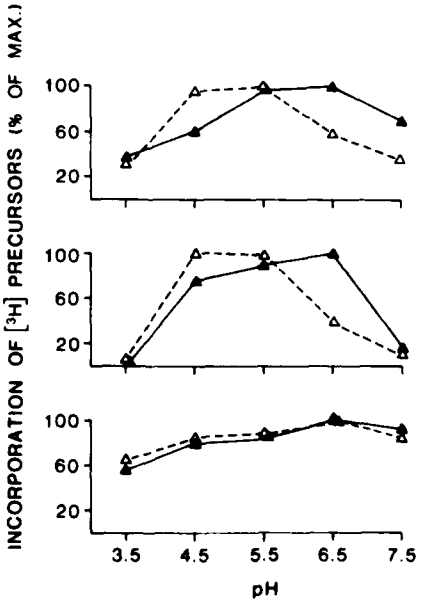

Fig. 4. Effects of pH on the in vitro synthesis of protein (top), RNA (center) and DNA (bottom) in *F. tularensis*. △, SCHU S4; ▲, LVS.

Macrophages obtained either from immunized animals or activated in vitro with endotoxin can effectively kill the virulent SCHU S4 strain. However, the addition of a weak base to the culture medium reverses the acquired microbici-

dal capacity of immune or endotoxin-activated macrophages for SCHU S4. One explanation for this observation is that a consequence of macrophage activation may be the acidification of phagolysosomes to pH values below 4.5, which is toxic to SCHU S4 (Fig. 5). Hyperacidification of macrophage phagolysosomes could result from the activation of the putative lysosomal proton pump (Schneider, 1979; Dell' Antone, 1979).

The validity of this concept remains to be established. At present, little is known about the mechanisms controlling phagolysosome pH or the effects of phagocyte activation on intravesicular H^+ concentrations. Nonetheless, the emerging observations in the *F. tularensis* system suggest that hyperacidification of phagolysosomes may be a requirement for the full expression of the microbicidal properties of activated macrophages.

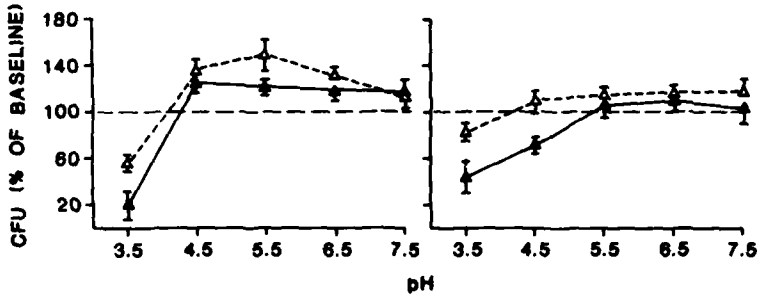

Fig. 5. Effect of pH and lysosomal extracts (100 μg lysosomal protein/ml) on growth of *F. tularensis* in vitro. △, no additions; ▲, lysosomes. (Left) SCHU 24, (right) LVS.

C. *Nonspecific activation*

In view of the macrophage's role in protection of the host against microbial infection, one should recognize that nonimmune activation of macrophages may be usefully manipulated for the prevention or treatment of acute infections. The nonspecific activation of mononuclear phagocytes in vivo is gaining attention as the number of agents capable of effective stimulation increases (Table 6).

While many biological materials such as endotoxin (Benacerraf and Sebestyn, 1957), BCG (Howard et al., 1959; Spencer et al., 1977), *Corynebacterium parvum* (Christie and Bomford, 1975), the methanol-extraction residue of tubercle bacilli (Gallily et al. 1977) and a number of lymphocyte factors (David, 1975) are capable of activating macrophages, few have been found suitable for use in the clinical situation. Levamisole, an antihelminthic agent, and poly(I)·poly(C), a polynucleotide, have been found to modulate the function of macrophages (Daughaday et al., 1977; Kelly, 1978). Neither, however, has been effective in treating systemic infection in animal models (Diamond, 1977; Morahan et al., 1977).

A more promising compound is glucan, an extract of yeast cell wall and the

TABLE 6

RETICULOENDOTHELIAL STIMULANTS

Stimulant	Reference
BCG (*M. tuberculosis*)	Howard et al. (1959)
Corynebacterium parvum	Christie and Bomford (1975)
Endotoxin	Benacerraf and Sebestyen (1957)
Estrogen	Bilbey and Nichol (1958)
Glucan	Riggi and Di Luzio (1961)
Killed bacteria	Di Luzio (personal communication)
Leishmania enrietti	Di Luzio (personal communication)
Lentinan	Maeda and Chihara (1971)
Levamisole	Kelly (1978)
Lipids, triolein	Stuart et al. (1960)
Lymphocyte factors	David (1975)
Methanol-extraction residue of tubercle bacilli	Gallily et al. (1977)
Muramyl dipeptide	Tanaka et al. (1979)
Mycobacterium phlei	Di Luzio (personal communication)
Poly(I)·poly(C)	Daughaday et al. (1977)
Pyran copolymer	Kapila et al. (1971)
Tilorone	Munson et al. (1972)
Tuftsin	Tzehoval et al. (1978)
Ubiquinones (coenzyme Q)	Block et al. (1978)
Zymosan	Benacerraf and Sebestyen (1957)

active component of zymosan (Riggi and Di Luzio, 1961). A potent stimulator of the reticuloendothelial system (Di Luzio, 1976), glucan increases the proliferation and phagocytic activity of macrophages in liver, lungs and spleen. More important, however, is the observation that glucan activation of macrophages and macrophage-related events can successfully moderate lethal infections. Glucan has been used successfully in animal studies to ameliorate the outcome of *Staphylococcus aureus* infection (Kokoshis et al., 1978; Di Luzio and Williams, 1978), tularemia and melioidosis (Reynolds et al., 1980). Resistance to viral infections such as Rift Valley fever (Reynolds et al., 1980) and mouse hepatitis (Williams and Di Luzio, 1980) is also increased. The enhanced resistance of glucan-treated animals to infection is believed to arise from enhancement of the microbicidal and immunological functions of macrophages. The potential exists, therefore, for augmenting host resistance to infection by nonspecific means. Thus, it is conceivable that this approach may be also applied in a compromised host in order to enhance its own cellular defenses against an infectious process.

Note

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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